CEPHALOSPORINS IN BONE CEMENT
STUDIES IN VITRO AND IN VIVO

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The properties of cephaloridine, cephalaxin, cefuroxime and cephalothin were examined in vitro after the incorporation of each of them with CMW Type 1 bone cement. When bathed in buffer at body temperature, cement containing cephaloridine, cefuroxime or cephalothin lost antibacterial activity after thirty-five to eighty-five days whereas with cephalaxin there was still activity after 185 days. Antibiotic passed rapidly out of the cement into the buffer for two days after immersion, then more slowly for some weeks; a total of between 23 and 32 per cent of antibiotic in the cement was eventually recovered from the bathing fluid. Hand mixing of dry antibiotic powder and cement powder resulted in an even distribution of the antibiotic, the mixture being stable when stored at 4 degrees Celsius.

In a group of nine patients undergoing total hip replacement, 500 milligrams or 1 gram of cephaloridine was mixed with each packet of Simplex cement. All the urine passed by the patients, and the drainage fluid removed from the wound for the first forty-eight hours after operation, were collected and the amount of cephaloridine was assayed. Urine was collected from some patients for up to fourteen days after operation. Cephaloridine gradually leached out of the cement in vivo, and was subsequently found in the urine and drainage fluid in generally similar amounts. Between 1.6 and 3.6 per cent of antibiotic implanted with the cement was recovered during the period of study, most of it during the first forty-eight hours after operation.

Many workers have confirmed that a wide spectrum of organisms, Gram-positive and Gram-negative, aerobic and anaerobic, can cause deep infection following total joint replacement (Fitzgerald et al. 1973; Kamme et al. 1974; Benson and Hughes 1975; Hunter and Dandy 1977).

The source of the infection may be air pollution or direct contamination at the time of operation, or a bacteraemia. Charnley and Eftekhar (1969) have introduced, with excellent results, the concept of ultra-clean air to reduce the incidence of infection after prosthetic replacement. Alternative techniques include the use of systemic antibiotics (Ericson, Lidgren and Lindberg 1973; Coventry et al. 1974; Hughes, Dash, Benson and Field 1978).

In 1970, Buchholz and Englebrecht introduced the idea of adding an antibiotic to bone cement to prevent deep infection from developing after primary joint replacement. For deep-seated infections to be prevented by an antibiotic in bone cement the preparation needs to be bactericidal for Gram-positive and Gram-negative organisms, whether aerobic or anaerobic. The antibiotic also needs to be stable during the exothermic hardening process, to have no effect upon the mechanical stability of the cement, to diffuse out slowly, retaining primarily a local effect, and to offer minimal risk of allergic or other side-effects.

There have been several studies of the suitability of antibiotics for incorporation into bone cement in recent years. Medcraft and Gardner (1974) studied fusidic acid because of its low allergenic and toxic potentials; it is not effective, however, against Gram-negative bacteria. Levin (1975) tested a wide range of antibiotics mixed into Simplex P bone cement and, although some were both broad-spectrum and bactericidal, no effective inhibition of Gram-negative organisms could be detected. All the antibiotics retained their activity through the hardening process but long-term retention of activity varied. Picknell, Mizen and Sutherland (1977) studied the release of various penicillins and concluded that the pattern of release was consistent with the leaching of antibiotic from or near the surface of the bone cement. Elson et al. (1977a, b) have continued the work of Buchholz and Englebrecht (1970) and of Buchholz and Gartmann (1972) by studying in vitro and in vivo the release of gentamicin from Palacos.

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cement. They confirmed Buchholz's original claims and showed that antibiotics mixed into bone cement can effectively control infection in animals.

The purpose of our paper is to report the behaviour in the laboratory of several cephalosporins in bone cement and of cephaloridine added to the cement used for nine patients undergoing total hip replacement. Cephalosporins were chosen for study because of their good activity against the majority of organisms encountered in deep infections of the hip.

MATERIALS AND METHODS

*In vitro studies.* Discs of non-radio-opaque cement with antibiotic were prepared by hand mixing 200 milligrams of sterile cefaloridine, cephalexin, cephalothin or cefuroxime with 1.8 grams of dry CMW Type 1 (CMW Laboratories Limited, Blackpool) bone cement powder before adding 1 millilitre of the liquid polymer. The mix was spread over a sheet of thin plastic with ninety-six holes each of 6 millimetres diameter formed from a Cooke's sterile microtitre tray with its cups cut away. When set, the resulting 6-millimetre discs, each 1.5 to 2.5 millimetres deep and weighing approximately 50 milligrams, were pushed out of the plastic. Thus standard discs containing 10 per cent dry weight of antibiotic were obtained.

The stability of the antibiotics in hardened cement, when bathed in fluid at body temperature and when kept dry, was studied. The discs were placed in buffer that was maintained at 37 degrees Celsius and changed at weekly intervals, or they were stored over silica gel at 37 degrees Celsius. Discs were removed at intervals and assayed for percentage potency by the agar diffusion technique. The assay organisms were Bacillus subtilis NC1B 8533 for cefaloridine and cephalothin, Bacillus subtilis MB 325DR for cefuroxime, and Sarcina lutea NC1B 8533 for cephalalexin. The agar used for assaying cefaloridine, cephalothin and cephalalexin was the same as that commonly used for penicillin but for cefuroxime the agar was Factor B with 0.5 per cent sodium chloride. Oxid sensitivity discs of the appropriate cephalosporin were included on all assay plates; the potencies of the cement discs were related to these and then expressed as a percentage of the original potency.

Discs containing 10 per cent antibiotic were also stored at 4 degrees Celsius in buffer that was changed daily and assayed for antibiotic content by the agar diffusion technique described above. This gave some indication of the concentrations of cephalosporin that might be achieved in the tissues surrounding a prosthesis.

The efficiency of hand mixing the antibiotic with the cement powder was investigated. Aliquots of 1.8 grams of cement powder were hand mixed with 200 milligrams of cefaloridine, cephalexin or cefuroxime and stored at 4 degrees Celsius. Samples of each mix were examined at intervals for a period of twenty-four weeks. The variations between the discs were noted and the stabilities over the period compared.

*Clinical studies.* For nine patients (five women, four men) who were undergoing total hip replacement, cefaloridine was added to Simplex P (Howmedica, London) cement powder. Emphasis was laid on thorough mixing of the antibiotic into the cement powder by stirring with a spoon for at least five minutes before adding the polymer. Cefaloridine, 500 milligrams per pack, was used for six of these patients; for the other three, a pack with 1 gram cefaloridine was used. The femoral component of the prosthesis was fixed using one pack, the acetabulum with another. The dry antibiotic powder was mixed with the cement in the operation theatre immediately before adding the liquid hardener. The amount of cefaloridine *in situ* was estimated by weighing the unused cement.

All the urine voided by the patients was collected six-hourly for the first forty-eight hours after operation. When the results were available from the first few patients, additional urine collections from subsequent patients were made twenty-four hourly from the third day to the fourteenth day. The urine was stored initially at 4 degrees Celsius, the volumes were measured and aliquot samples were then stored at -20 degrees Celsius until they were assayed.

All patients had suction drainage for the first twenty-four to forty-eight hours. Drainage fluid was collected and stored initially at 4 degrees Celsius. After measurement of the volumes, representative samples were stored at -20 degrees Celsius.
Assays were performed by large-plate agar diffusion, using a spore suspension of Bacillus subtilis NCIB 8533 as inoculum for the plates. The amount of cephalexin excreted in each sample of urine and drainage fluid was then calculated.

Patients were closely observed during their in-patient stay. They were examined in the follow-up clinic one, four and twelve months later.

RESULTS

In vitro studies. The antibacterial activity of each of the four cephalosporins in hardened cement is summarised in Figure 1. All four were effective against Gram-positive and Gram-negative organisms at concentrations of 1.25 to 10 per cent dry weight.

The discs stored over silica gel retained the activity of all four cephalosporins for at least thirty-nine weeks. In buffer at body temperature, discs of cement containing cephalexin showed no antibacterial activity after seven weeks, those with cefuroxime or cephalothin had no activity after twelve weeks but those containing cephalaxin still possessed good antibacterial activity after twenty-six weeks. The results are shown in Figure 2. The eventual loss of antibacterial activity was at least partly explained by passage of the cephalosporin into the buffer (Fig. 3). A large proportion of drug was released into the buffer during the first forty-eight hours although antibiotic continued to pass slowly out of the cement for some weeks. The recovery rate for the cephalosporins was far greater than that reported by Buchholz and Englebrecht (1970) in their tests with erythromycin and gentamicin.

The results in Table 1 indicated that an even distribution of the antibiotics through the cement could be achieved by meticulous hand mixing. The cephalosporins were each stable for at least twenty-four weeks when mixed with the dry cement powder and stored at 4 degrees Celsius.

Clinical studies. The age of the patients ranged from fifty-four to seventy-one years. Urine samples were collected six-hourly from all nine patients for the first forty-eight hours after operation and twenty-four-hourly thereafter from five of them. Collections from one of these five (Case 5) were discontinued on the fourth day when she received ampicillin for a chest infection; collections from the other four continued until the fourteenth day. The samples taken over the first forty-eight hours from one patient (Case 9) were lost, as was one six-hourly sample from another patient (Case 1).

Table 1. Assayable antibiotic content in bone cement powder after incorporating 100 milligrams per gram of three cephalosporins by hand mixing

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Average concentration of five samples (mg/g)</th>
<th>Deviation from theoretical mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average (per cent)</td>
<td>Range (per cent)</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>116.4</td>
<td>23.6</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>113.2</td>
<td>14.8</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>105.2</td>
<td>6.0</td>
</tr>
</tbody>
</table>

The concentrations of cephalexin in the urine lessened rapidly after the first twelve hours, a decrease that was partly because of the diuresis which began at this time in most patients. Figure 4 shows, for the seven patients from whom complete samples were available, the amount of cephalexin excreted in the urine during the first forty-eight hours after operation, the mean amount being 7.8 milligrams. The considerable variation between patients did not seem to be related to the concentration of antibiotic in the cement. With all patients, however, the amount excreted decreased markedly after twenty-four hours. As might be expected, there was poor correlation between the amount excreted in forty-eight hours and the volume of urine \( r=0.185 \). After the first forty-eight hours the amount of cephalexin excreted in the urine was very small: from two patients (Cases 3 and 4) none was found; in another patient (Case 9), 0.7 milligram was
obtained on the third day; from another (Case 1), 2 milligrams was found on the sixth day; from Case 5, 0.3 milligram was recovered on the third and fourth days. The mean amount excreted during the period of observation was 8.2 milligrams (Table II).

The concentration of cephaloridine in the drainage fluid ranged from 6.4 to 26.0 micrograms per millilitre (mean 17.0 micrograms per millilitre). It varied among patients and did not correlate with the volume of the fluid ($r=0.014$). The mean amount of antibiotic excreted in the drainage fluid was 8.7 milligrams, similar to that excreted in the urine (Table II).

In four patients the unused cement was weighed. The amounts of cephaloridine excreted in the urine and drainage fluid were expressed as a proportion of cephaloridine actually inserted with the prosthesis. The values ranged from 1.6 to 3.6 per cent with a mean of 2.9 per cent (Table II).

Three patients developed minor superficial infections of the wound caused by cephaloridine-sensitive Staphylococcus epidermidis. In one patient (Case 1), a discharge developed on the seventh day after operation; in the two others (Cases 2 and 6), a discharge developed after they had left hospital. All recovered without complications. No deep infections and no adverse effects have developed in this small group of patients during the one year of follow-up since operation.

![Fig. 4](cumulative_excretion.png)

**Cumulative excretion of cephaloridine in the urine of patients after mixing 500 milligrams (open symbols) or 1 gram (closed symbols) per cement pack.**

### DISCUSSION

Infection after total hip replacement is generally considered to result from bacterial seeding of the site at the time of operation. Late infections have been shown to be associated with a transient bacteraemia quite separate from the operation (Hall 1974).

A bacteraemia may occur early. In the experimental animal such a bacteraemia has caused organisms to be lodged in the bone cement (Elson et al. 1977b), and it would therefore seem logical to have the cement impregnated with antibiotic for as long a period after operation as possible.

The incorporated antibiotic should be capable of inhibiting the growth of any bacteria reaching the surface of the cement. To do this, the antibiotic must, while remaining biologically active, leach out of the cement slowly and then diffuse through bone. The cephalosporins are water-soluble and bone cement is a porous structure. Diffusion through the cement could occur, dependent on the concentration gradient and the sizes of the molecules and of the pores in the cement. Once in bone, diffusion occurs from the endosteal to the periosteal surface, passing through the bone fluid space (Hughes, Davies, Khan and Kelly 1978). The structure of the cement and the molecular size of the antibiotic are therefore important factors in the diffusion through bone cement.

Our in vitro studies indicated that the cephalosporins we tested were able to prevent the growth of bacteria near the surface of the impregnated cement and that they retained activity for several weeks under moist conditions at body temperature. Although, under these conditions, cephaloridine lost bactericidal activity more rapidly than the other cephalosporins available as sterile powder, it has the advantage over cephalothin in that it is not metabolised in vivo. Moreover, cephaloridine is excreted almost solely by the kidney; after parenteral administration virtually the whole dose is found in the urine during the ensuing twenty-four hours (Muggleton,
O'Callaghan and Stevens (1964). When the clinical studies were being done it was more readily available than cefuroxime.

The fact that the patterns of elution were similar in vitro and in vivo was reassuring. The likelihood is, therefore, that antibacterial levels of cephaloridine will be present in the cement for some time after operation, helping to sterilise the bone and surrounding tissues.

If a bacteraemia occurs, the presence of an antibiotic in effective concentrations at the interface between bone and cement will be a benefit. Bacteria presumably reach bone by the Haversian system and, like minerals, are subject to the same process of transcapillary exchange—that is, passive diffusion (Davies, Bassingthwaighte and Kelly 1976; Hughes et al. 1977).

There seems no possibility that significant antibacterial effect will remain to prevent late seeded infections at two to three years. Nor do antibiotics in cement prevent superficial wound infections although, because of the concentration in drainage fluid, they may reduce the risk of infection in any haematoma.

We have previously advocated the use of three 1-gram injections of cephaloridine as a prophylactic measure at the time of operation on a joint (Hughes, Dash, Benson and Field 1978) when the risk of infection is greatest. By adding antibiotics to cement, further cover may be acquired to prevent any bacteraemia that may develop in the first few weeks from initial colonisation of the bone cement.

Our conclusions are that the cephalosporins, which are effective against most of the organisms encountered in deep infection of the hip, can be incorporated into bone cement and that the in vivo kinetics of at least one of these antibiotics, cephaloridine, mirrors its in vitro behaviour.

REFERENCES


